12. Chemical Methods

Crude protein – improved Kjeldahl method, copper catalyst modification

(American Association of Cereal Chemists – AACC-Method 46-11)
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1 Scope

Applicable to nitrate-free samples; also to flour, wheat and other grains, cereal adjuncts, yeast foods, and animal feeds.

2 Apparatus

2.1 Kjeldahl flasks, Pyrex or equiv., 800 ml capacity; used for both digestion and distillation (distn.)

2.2 Digestion heaters, 600 W (more or less, depending on voltage). Heater unit should bring to vigorous boil 250 ml water at 25°C in 5 min with hot burners.

2.3 Digestion unit; consists of electric heaters, large lead tube, and plastic fume stack with suction fan capable of exhausting toxic fumes to outside air.

2.4 Distn unit (see footnote); to consist of Iowa State-type connecting bulbs (traps) 36 x 100 mm, Pyrex glass condenser tubes, pure gum-rubber stoppers and tubing, electric heating units (600 W), and condenser tubes capable of being kept cool with adequate amts of cool water during distn and with thermo-water control on stills. Upper ends of bulbs connect with high-quality rubber tubing to condenser tubes; lower ends with rubber stoppers to 800 ml distn flask. Lower ends of condenser tubes have rubber-connected glass or polyethylene tubes that lead to:

2.5 Receiving bottles or flasks, 300 ml capacity.

2.6 Proper burettes for dispensing (a) conc. H_2SO_4, (b) caustic soda, (c) 0.1 N H_2SO_4, and (d) 0.1 N NaOH. (See 6.1)

3 Reagents

3.1 H_2SO_4, conc. (95-98%, nitrogen-free; sp gr 1.84).

3.2 Catalyst; 15 g potassium-sulphate, 0.04 g anhyd. $CuSO_4$, 0.5-l.0 g Alundum granules (see 6.2).

3.3 Anti-bumping agent; zinc metal, 20mesh; pumice stone or Alundum, 8-14 mesh. Can be combined in catalyst mixt.

3.4 NaOH, pellets or soln., nitrate-free. For soln, dissolve approx. 450 g solid NaOH in 1 L water. Sp gr of soln should be 1.36 or more.

3.5 Methyl red indicator. Dissolve 1 g in 200 ml alc. (95 %). Other indicators may be used satisfactorily. (See 6.3).

3.6 Std NaOH, 0.1 N. Weigh 73 g NaOH per 18 L water and stdze. May be stdze by titration against pure acid potassium phthalate (NBS SRM for acidimetry 84 is recommended) dissolved in CO_2-free water, using phenolphthalein as indicator; 0.5108 g will neutralise 25 ml 0.1000 N NaOH. Other recognised stdze methods may be used. (See 6.4).

3.7 Std H_2SO_4, 0.1 N. Add 50.4 ml H_2SO_4 (reagent grade, sp gr 1.84) to 18 L water. Titrate against std NaOH and adjust as necessary, using methyl red as indicator. Other recognised stdze procedures may be used. (See 6.4 and 6.5).

4 Procedure

4.1 Weigh quickly and accurately well-mixed and finely ground sample. Bread, 2 g prepd by Method 6205; yeast foods, 0.5 g; wheat and other grains, feeds and feed stuff, 1.0 g. Place in digestion flask. (Sample may be placed in nitrogen-free paper to prevent clinging to sides of flask.) Add catalyst (reagent 3.2) and 20 ml conc. H_2SO_4 to flask. Add addnl 1.0 ml H_2SO_4 for each 0.1 g fat or 0.2 g other organic matter if sample wt is over 1.0 g. Heat flask at specified rate until dense white fumes clear bulb of flask, swirl gently, continue heating addnl 90 min. (See Note 6.2) Remove and cool but do not allow to crystallise.

4.2 Add 25 ml std acid to 300 ml bottle or flask, dil to 50ml, add indicator, and immerse tip of condenser tube in this receiver soln. Add 250-275 ml tap water to cool digestion flask. Add 2-3 drops of tributyl citrate to distn flask to reduce foaming; add another 0.5-1.0 g Alundum granules. Gently add 50 ml conc. NaOH, connect to condenser with tight-fitting rubber stopper, and swirl. Boil until all ammonia has dist. (at least 150 ml of distillate) and then set receiving bottle down so that condenser tube is completely drained.

4.3 Titrate distillate with std NaOH soln to neutrality, using methyl red indicator.

4.4 Run blank detn using all ingredients except sample.

5 Calculations

$$\%\ \text{Protein} = \frac{(B - S)\ x\ N\ x\ 1.4007\ x\ f}{\text{sample weight (g)}}$$

where

B = ml alk back-titration of blank
S = ml alk back-titration of sample
N = normality of alk
f = 5.7 for bread, wheat, and wheat flour, f = 6.25 for other grains, f = 6.38 for milk products, and f = 6.25 for samples of unknown source

6 Notes and Precautions

6.1 In routine testing of large no. of samples, use large dispensing burettes for conc. acid and alk and for receiver acid, which may contain indicator.

6.2 As a catalyst, copper sulphate is recommended as less hazardous than either mercury or selenium, or their compounds. Kane (Ref. 4) stresses that specific parameters of time, heat input, and salt-acid ratio are important. Adequate exhaust ventilation must be provided in digestion-distn area.

6.3 Mixed indicator consisting of 0.75 g methyl red and 0.625 g Guinea green per L or 0.75 g methyl red and 0.5 g methylene blue (Ref. 9) dissolved in 300 ml alc may be used. Any indicator used should have sharp end point and distinct color change.

6.4 Rodkey (Ref. 9) has successfully applied tris (hydroxymethyl) aminomethane as a convenient primary std for direct stdze of acid solns.

6.5 Reeder and Patton (Ref. 8) suggested use of reagent-grade sodium acid sulphate ($NaHSO_4 \bullet H_2O$) in water to make std soln equiv. to std H_2SO_4; 13.81 g/L will give 0.1000 N soln.

6.6 To check entire protein method, digest 0.1 g pure ammonium oxalate (monohydrate) with 1 g pure sugar using regular procedure. Resulting protein should be 11.24 % calcd as follows: $(NH4)_2C_2O_4 \bullet H_2O$ with mol wt of 142.12 contains 28.016 g nitrogen or 19.713 %; 19.713 % x factor 5.7 : 10 (0.1g sample) = 11.24 % protein.

7 References

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